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Saltgrass (*Distichlis spicata*) – A New Salt-Resistant Forage Crop for the Reclamation of Salinized Soils

Report from July 2001 to November 2002

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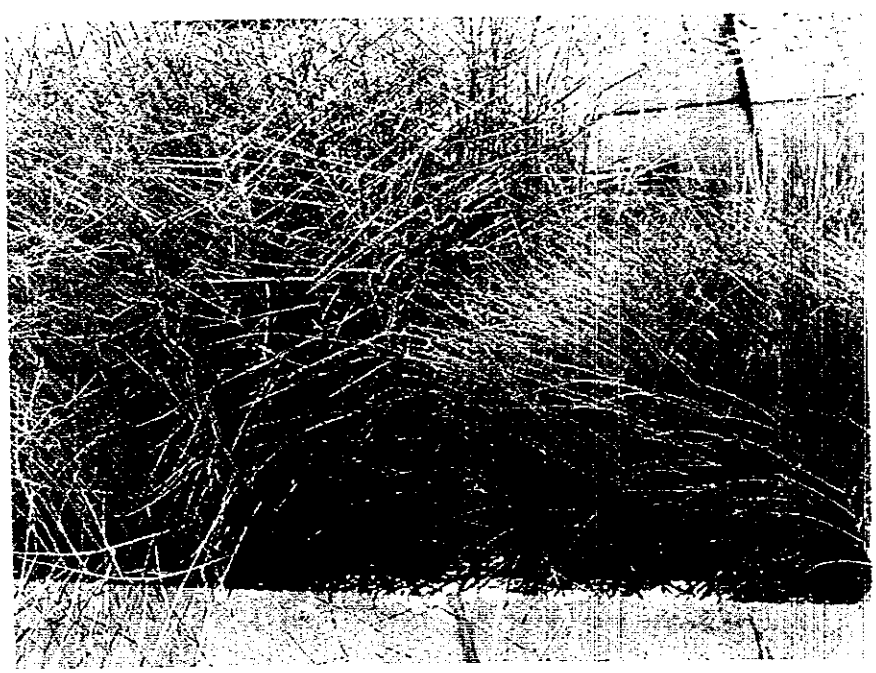

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Abstract

This report summarizes research activities made during the first year of the AID/CDR/CAR project (CA20-058). The objectives this year were to select saltgrass accessions from our collection in Israel that will be suitable as forage at saline soils and water. Criteria were growth performance under saline conditions, leafiness and other forage quality parameters. At first, we characterized our collection through aspects of genetic variability, leafiness and morphology, and basic forage quality. Secondly we examined the performance of our accessions at different salinity levels. It may be already stated, that accessions from the southern Atlantic coast of the US appeared to have the best potential, although other origins should mot be discarded at this stage. The basic forage quality of saltgrass appeared quite high, as related to the common forage crop alfalfa. Yet, forage quality parameter should be further evaluated at field experiments and under saline conditions. Six saltgrass accessions have been already transferred to Turkmenistan for propagation and examination of their performance at the harsh conditions of that country. Two field experiments were established to examine saltgrass response to fertilizer level and to salinity gradient. Results are expected at the end of 2003 autumn.

Introduction

This project is aimed to examine the possible use of saltgrass (Distichlis spicata) as a forage crop to be grown at the early stages of saline soil reclamation in the Aral Sea Basin, in Turkmenistan. Following decades of land abuse, large areas of former fertile soils have become saline to a level that does not permit further agricultural use. The ultimate way to reclaim those lands is to leach the soil with water at lower salinity. It is proposed here, that that leaching process will be accompanied by forage production, which can provide some income. To do so, a salt-resistant, productive, and valuable crop species should be used. Saltgrass is a wild grass species that origins in the continental America. It can be found in the tidal areas of most western, southern and eastern coasts of the US, as well as in inland saline, temporary wet depressions in North and South America. There is evidence for saltgrass use for livestock feeding, however, this species was not domesticated neither selected for modern agricultural uses. It is our aim, therefore, to characterize the species for further selection and to evaluate its potential for forage and other uses.

In 1998, we established a wide collection of saltgrass accessions brought from many different regions of the US and some South American accessions as well. This collection is used as the basis for a long process of selection and domestication of saltgrass. It should primarily provide us initial estimates of the potential buried in this species and it might also point out specific regions that have better potential than others as good sources of saltgrass.

The objectives of the research in year 2001-2002 were: 1. to characterize our saltgrass collection in Israel and to select those accessions that display potential suitability as forage at various saline conditions. 2. to transfer selected ecotypes to Turkmenistan, and 3. to establish field experiments for the next years in both countries.

Results

Characterization of the saltgrass accessions

In 1998, long before the approval of the present AID/CDR/CAR project, we had established a wide collection of saltgrass ecotypes that were brought from various and different eco-geographical regions in the US and South America. After the required quarantine period, plants were grown in pots in the Institutes for Applied Research, BGU, in Beer Sheva. About 40 genotypes (accessions), representing most of the regions of collection, were examined to characterize the present collection. We characterized and sorted the accessions for their morphological traits and the genetic diversity within the collection was estimated using RAPD. A preliminary evaluation of the feed value of each accession was evaluated. These examinations allowed us to identify those regions with potential plant material for further collection tasks. Furthermore, we could select potential accessions for further characterization and for more applied research. Some of the selected accessions were examined for their response to various salinity levels of the growth medium.

Plant material

The origin of the accessions in the collection is shown in Table 1.

Table 1: Sampling sites of saltgrass (*Distichlis spicata)* ecotypes.

North America						
Ecotype	Country /State	County / Location	Inland/ Seashore	Latitude (°N)	Longitude (°W)	Notes
CT1,CT3	Connecticut	New London / Barn Island	Seashore	41°32'15	71°86'86	Tidal marsh on seashore
CT2	"	New London / near Mystic	Seashore	41°32'03	71°91'71	Tidal marsh on seashore
DE1-2	Delaware	Sussex / near Lewes	Seashore	38°80'16	75°23'32	Tidal marsh on seashore
DE3						UDE collection, origin unknown
GA1-4	Georgia	McIntosh / Sapelo Island	Seashore	31°42'21"	81°24'17	Tidal marsh on seashore
AL1	Alabama	Mobile / Deer River	Seashore	30°31'52	88°06'30"	Tidal estuary
AL2	"	Mobile / Dauphin Island	Seashore	30°15'26"	88°07'33"	Tidal marsh on seashore
AL3	"	Mobile / Heron Bay	Seashore	30°20'57"	88°07'48"	Tidal marsh 1km from seashore
AL4	"	Mobile / Heron Bay	Seashore	30°21'44"	88°07'39"	"
AZ1	Arizona	Cochise/ Willcox playa	Inland	32°09'51"	109°55'38"	Seasonally inundated depression, desert
AZ2	Arizona	Cochise/ Willcox playa	Inland	32°10'31"	109°55'38"	"
AZ3	Mexico	Colorado river delta	Inland			*Distichlis palmerii*
UT1	Utah	Goshen	Inland	39°57'00"	111°53'31"	Inland saline marsh, seasonally inundated
UT2	"	Big Salt Lake	Inland	-	-	Inland saline marsh, seasonally inundated
UT3	"	Vernon	Inland	40°04'50"	112°24'47"	River bank, seasonally inundated
UT4	"	Jordan River	Inland	40°50'06"	111°56'39"	River bank, seasonally inundated
CA1	California	/ Mendota	unknown	-	-	Origin uncertain
CA2	"	Riverside / Salton Sea	Inland	33°18'47"	115°36'44"	On shore of salty lake
CA3	"	Kings / Tulare Lake-bed	Inland	35°58'05"	119°56'34"	Drainage ditch banks
CA4	"	S. Luis Obispo/Soda Lake	Inland	35°08'38"	116°06'16"	Seasonally inundated depression
CA5	"	Fresno / Tranquillity	Inland	36°39'45"	120°14'24"	Drainage ditch banks
CA6	"	S. Luis Obispo/Morro Bay	Seashore	35°20'50"	120°50'34"	Tidal estuary
CA7	"	Merced / Hartley Slough	Inland	37°16'08"	120°30'50"	Seasonally inundated depression
CA8	"	San Joaquin	Inland	36°34'21"	120°11'13"	Drainage ditch bank
CA9	"	San Diego / Mission bank	Seashore	32°45'42"	117°12'22"	River bank
CA10	"	Tulare / Alpaugh	Inland	35°54'17"	119°29'51"	Drainage ditch bank
CA11	"	Monterey/Elkhorn Slough	Seashore	36°50'27"	121°44'37"	Tidal estuary
CA12	"	Kings / Kettleman city	Inland	36°03'50"	119°58'03"	

South America						
Ecotype	Country /State	County / Location	Inland/ Seashore	Latitude (°S)	Longitude (°W)	Notes
Chi1	Chile	Tamarugal	Inland	19°05'	68°30'	Precise location unknown
Chi2	Chile	Juta Valley	"	20°30'	67°20'	"
Arg1	Argentina	Trelew Chubut	"	44°05'	67°25'	"
Arg2	Argentina	Chinchina La Rioja	"	29°15'	65°40'	"

As shown in Table 1, the saltgrass collection can be divided into groups according to the type of their geographical or eco-geographical origin. We examined the differences among accessions within each group and among groups in order to identify linkage between interesting traits of saltgrass plants and their origin.

Morphological analysis

Pot-grown plants (genotypes) were visually characterized for selected morphologic parameters. Canopy growth was ranked from 1 to 5 for vigor (weak to strong), shoot direction (crawling to erect), and density (low to high). Leaf features were sorted according to: color (bright to dark blue green, 1-5); the angle between the leaf and the shoot - 1 (0-30°), 2 (30-60°), and 3 (60-90°); leaf length – 1 (<1cm), 2 (<3cm), 3 (<5cm), 4 (<8cm) and 5 (>8cm); leaf width – 1 (narrow) and 2 (wide); leaf texture – soft to rigid (1-5).

PCA analyses of eight morphological parameters were conducted to reveal clustering patterns of saltgrass accessions according to geographical or eco-geographical sorting (Fig.1). In the geographical sort, three clusters were obtained: 1) All saltgrass accessions from GA and AL; 2) Californian accessions; and 3) South American genotypes (Fig. 1A). In that clustering pattern, only the GA and AL group clearly segregated from the other accessions, sharing common canopy features as strong vigor, erect shoots, and relatively low density as well as relatively longer, wider, and soft-textured leaves (Fig. 2). The other North American accessions were scattered with the Californian and the South American groups (Fig. 1).

Two genotypes with unclear origin, CA1 and DE3, and genotype AZ1, displayed uncommon morphological traits, and were, indeed, located in the PCA far from all other saltgrass accessions. On the other hand and in spite of being a different species (*Distichlis palmerii*), AZ2 was located in the PCA very close to the large North- and South American cluster of *Distichlis spicata* (Fig.1). Albeit the clear segregation of

the GA and AL group, which was consisted of seashore genotypes, the coastal accessions in general did not share any clear-cut morphological traits that could distinguish between them and inland accessions, as indicated by the mixed scattering of these two groups in the PCA (Fig. 1B).

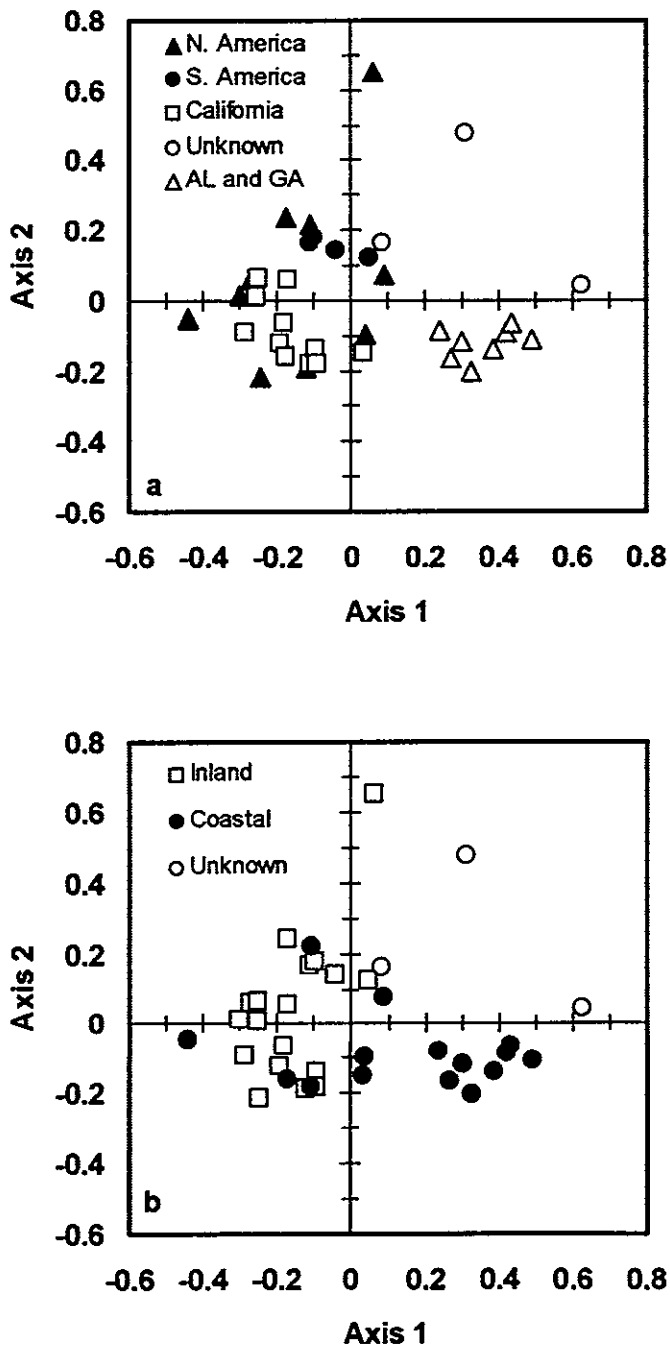


Figure 1. PCA analysis of morphological traits in a collection of 37 *D. spicata* genotypes. A. Genotypes are sorted according to geographic origin. B. Genotypes are labeled according to their eco-geographic origin (inland or seashore types).

Large phenotypic variation occurred among accessions, however, attempts to relate morphological traits to geographic or eco-geographic origin generally failed (Fig. 1). Only the GA and AL accessions clustered and segregated from the others, sharing some unique patterns of growth (Fig. 2). Californian, as well as South American accessions, clustered but could not be distinguished from other accessions in this respect (Fig. 1A). There were no morphological traits that could specify neither inland nor coastal accessions hence discrimination between those two groups on a phenotypic basis was ineffectual (Fig. 1B).

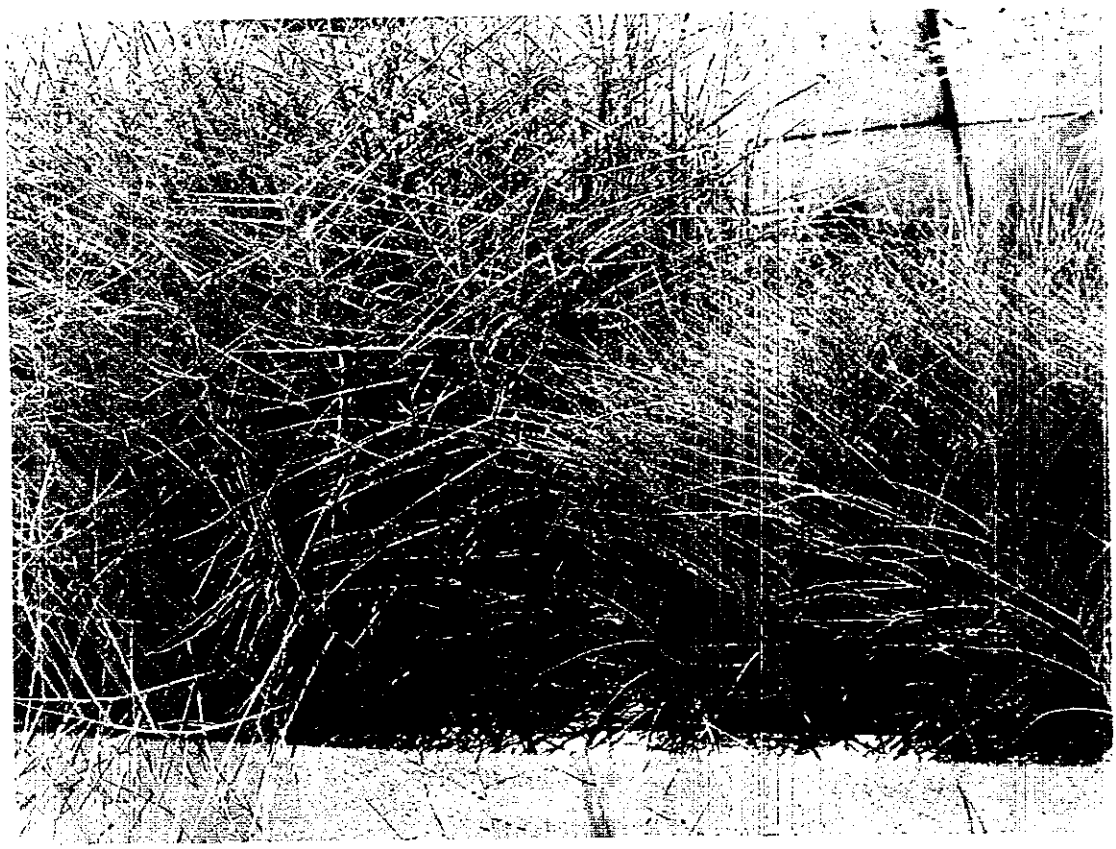


Figure 2. Pot-grown plants of saltgrass accessions GA2 (left) and Arg1 (right), demonstrating differences in traits such as vigor, density, shoot direction, leaf angle and size, and color.

Genetic Analyses

DNA was extracted from 0.5g fresh leaves by the CTAB method (Clark, 1997). Thirty ng of DNA were used as template for RAPD reactions. Twenty decamer primers (Kit D, Operon Technology, Alameda, CA) were screened using 25 µl PCR reaction. PCR conditions were as follows: 94^{0}C/60 sec, 40^{0}C/30 sec, 72^{0}C /180 sec (4 cycles); 94^{0}C /30 sec, 40^{0}C /45 sec, 72^{0}C /60 sec (30 cycles); 72^{0}C /8 min (1 cycle).

PCR products were loaded on a 2% agarose (Amresco, Oh.) gel and run at 80V/14 cm for 4 hours. In order to assess the reproducibility of the RAPD-PCR, DNA was extracted twice at different days from each genotype, using different working solutions. DNA from each extraction was then used as template in independent PCR reactions. Most of the amplified fragments obtained from the two replicates were identical and out of these, 70 different bands were selected for RAPD analysis and calculation of genetic distances. Each selected band was scored by for its presence (1) or absence (0). On this basis, a matrix involving all RAPD phenotypes was assembled. Since RAPD markers are dominant, we assumed that each band represented the phenotype at a single bi-allelic locus (Williams et. al, 1990). To obtain genetic distances values among *D. spicata* individuals, pair-wise distance matrices were computed based on Nei's (1987) unbiased Identities/Distances using the TFPGA software (Miller, 1997).

The relationships among RAPD phenotypes were assessed as follows: a dendrogram was constructed using the unweighted pair-group method with arithmetical average (UPGMA), by TFPGA software (Miller, 1997). PCA was exercised on the morphologic and RAPD results using the MVSP package (Kovach, 1999) in order to obtain a two-dimensional clustering pattern of *D. spicata* accessions using various geographical or eco-geographical parameters of sorting.

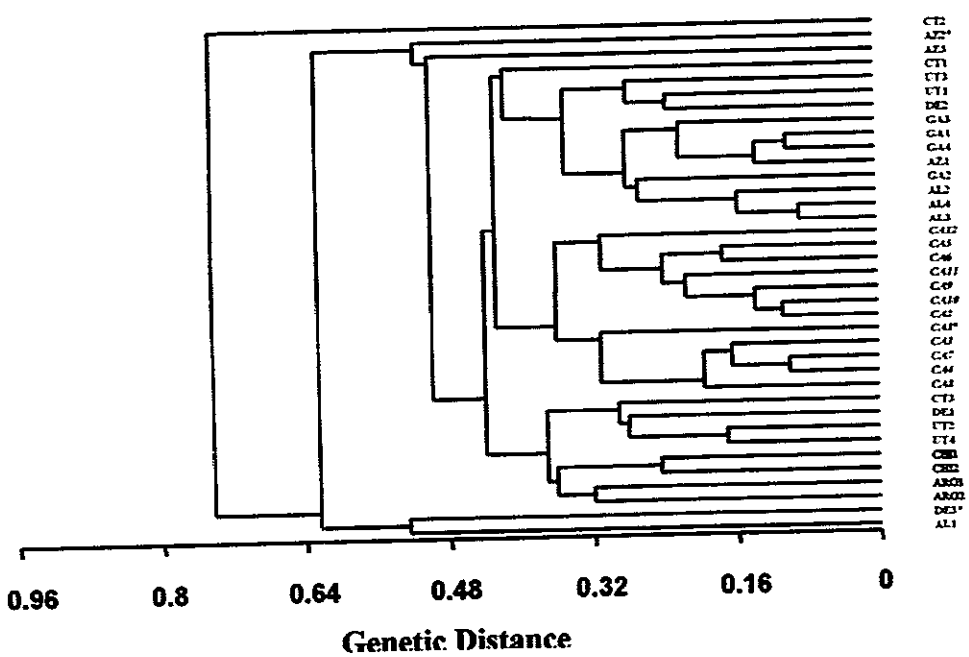
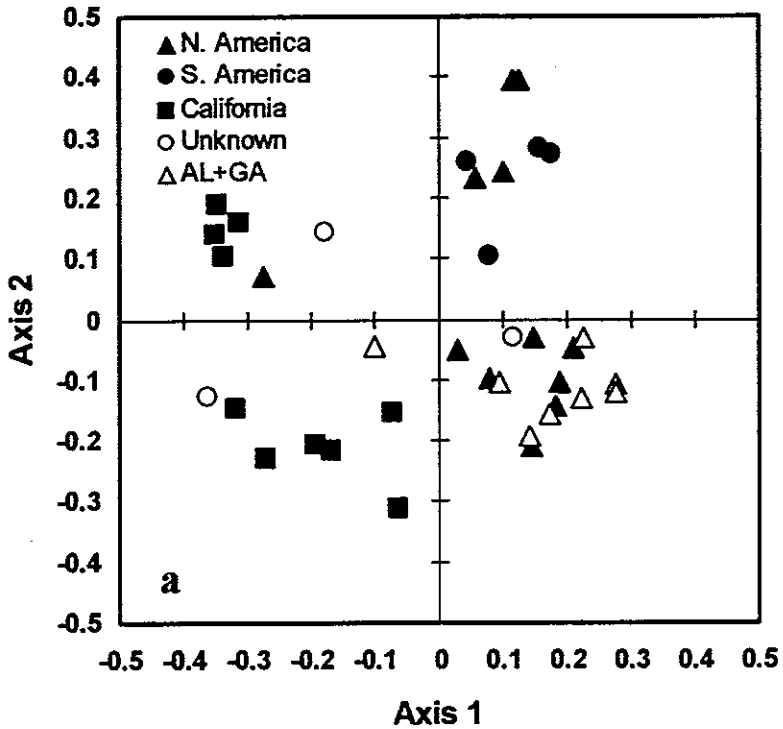


Figure 3. Cluster analysis (UPGMA) based on Nei's distances among 37 *D. spicata* genotypes. * indicates for a genotype of unknown origin. Bold letters indicate for N. American accessions (excluding CA), Bold-italic letters – Californian accessions, and regular letters – S. American genotypes.

With a few exceptions, the dendrogram clustered the genotypes into distinct groups according to a general pattern of their geographical origin (Fig. 3). Three major groups were distinguishable. The first included most of the genotypes collected from North American states, excluding California. Within that group, most AL and GA genotypes were clustered in two close subgroups. The second group comprised of Californian genotypes exclusively. The third group was divided into two subgroups, one of which consisted of all four genotypes from South America (Chile and Argentina), while the other included four North American genotypes (CT3, DE1, UT2 and UT4). Five individuals (DE3, AL1, CT2, AZ2 and AZ3) were not clustered within these three groups and the genetic distances among them were particularly broad. It should be mentioned that extensive genetic distance was displayed among the three CT individuals (Fig. 3), although geographic distances among them were extremely small (Table 1).

In order to gain some insight into the grouping of *D. spicata* individuals from our collection, a PCA was carried out, using RAPD band pattern as raw data (Fig. 4). The PCA reflected some of the relationships among genotypes already observed in the dendrogram (Fig. 3), since similar grouping of individuals could be detected. Clustering of individuals according to their geographical origin was detectable in some cases: the PCA axis2 clearly disjoined the Californian genotypes from most others. Axis1 parted South American individuals from the majority of North American genotypes, with the exception of CT3, DE1, UT2 and UT4 (Fig. 4A), as already observed in the dendrogram (Fig. 3). AL and GA genotypes clustered together (excluding AL1) but did not segregate from other N. American types (Fig. 4A).

On the other hand, saltgrass genotypes did not cluster according to their inland or seashore origin, as shown in Figure 4B. On the contrary, definite seashore ecotypes from Georgia, Connecticut, and Alabama were grouped together with absolute inland ecotypes from Utah and Arizona. Even in California, seashore and inland ecotypes were mixed.



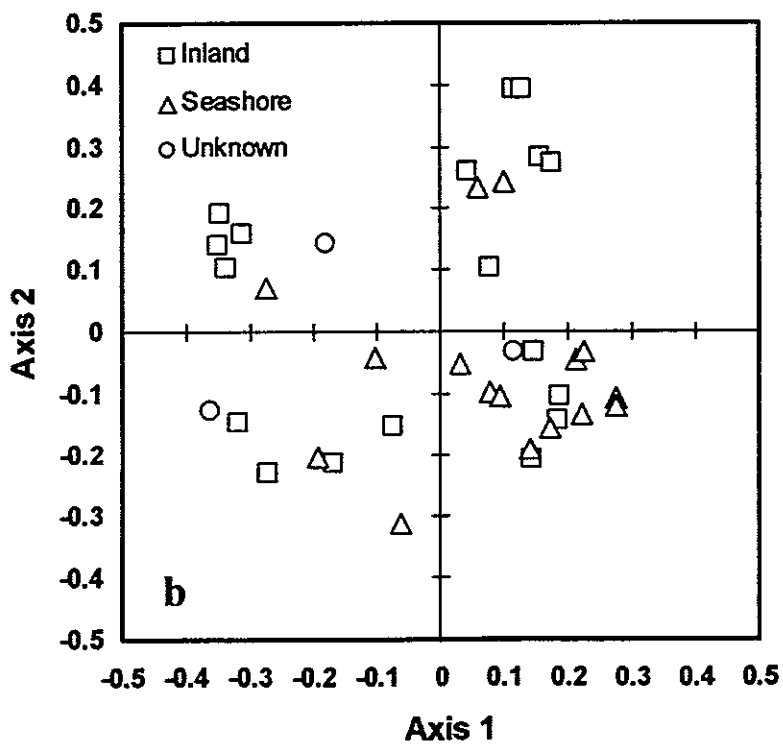


Figure 4. PCA analysis of RAPD results of 37 *D. spicata* genotypes. A. Genotypes are sorted according to geographic origin. Axis 1 extracted 13.7% of the variation and axis 2 extracted 11.3% of the variation. B. Genotypes are labeled according to their eco-geographic origin (inland or seashore types).

Evaluation of Feed Values

After reviewing the literature on determinants of quality in forage grasses, Marten (1989) concluded that leafiness (leaves to stems ratio) is the main determinant of forage quality, since leaf tissues contain relatively smaller amount of indigestible fibers compared to stems; the smaller the fiber-content the higher the feed value of the plant material (if other components such as proteins and ash are similar). Therefore, selection and breeding for quality should concentrate on improving leafiness of grass species. In previous investigation it was concluded that *D. spicata* is of good potential as a forage crop, however, types of better quality must be selected (Pasternak et al., 1993). The main problem was a low rate of leafiness. At these early stages of the project we select for the best genotypes of saltgrass.

Plants were grown in 10L pots with adequate water and fertilizer. Plants were harvested periodically according to size and developmental stage and leafiness was evaluated, ranking from 1 (low) to 10 (high). Samples of plant material were oven-dried to constant mass at 50°C and those used to determine dry matter content were oven-dried to 100°C. Nitrogen (N) content was determined by the Kjeldahl method and for ash by burning at 550°C (AOAC 1990). Crude protein was calculated as 6.25xN. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) are still at the process of determination according to Goering and Van Soest (1970). The difference between NDF and ADF will be designated as hemicellulose, and between ADF and ADL as cellulose.

Gross energy of the samples will be measured by bomb calorimetry (Gallenkamp, model CBB-370) using benzoic acid as standard (26,453 J/g, BCS-CRM No. 190n, Bureau of Analyzed Samples Ltd.). Metabolizable energy (ME) of the samples was estimated *in vitro* using the Hohenheimer Gastest (Menke, Raab, Salewski, Skingrass, Fritz and Schneider 1979). In this method the gas produced in anaerobic fermentation of substrate is used to predict the nutritive value. Rumen liquor and particulate matter was collected before morning feeding from two goats and two sheep fed on a *Distichlis spicata* diet; the liquor was homogenized, strained and filtered through glasswool. Incubation media was prepared as described by Menke et al. (1979). Samples, each 200mg dry matter, were incubated in triplicate in 100ml calibrated glass syringes in which 30ml of the incubation media were added. The glassware was kept at 39°C and flushed with CO_2 before use and mixture was kept stirred under CO_2 at 39°C. Gas production, as determined by the piston movement, was measured over

24 hours after correcting for gas production due to rumen fluid alone. Metabolizable energy (ME, kJ/g dry matter) is then calculated from the gas produced (GP; ml from 200g dry matter/ 24h) and crude protein (XP; g/kg dry matter) as:

$$ME = 2.20+0.13GP+XP.$$

Table 2. Preliminary evaluation of forage quality among saltgrass accessions, clustered according to their geographical origin. Alfalfa is used as a reference forage crop.

Geographical Origin	Leafiness (1-10)	Dry Matter (DM) (%)	Ash Content (% of DM)	Crude Protein (% of DM)	Metabolizable Energy (Sheep) (kJ/g DM)	Metabolizable Energy (Goat) (kJ/g DM)
Georgia	8.38±0.28	43.92±0.65	6.10±0.17	9.56±0.54	6.65±0.14	6.44±0.18
Delaware	5.60±0.57	43.15±3.44	7.15±0.08	13.03±0.78	6.44±0.31	5.45±0.23
Alabama	8.33±0.37	45.45±1.11	6.73±0.53	9.84±0.37	6.22±0.19	5.75±0.39
Connecticut	7.67±0.41	43.68±0.87	7.24±0.37	14.27±0.96	5.98±0.48	4.64±0.88
California	6.33±0.91	42.85±3.23	6.78±1.18	11.96±1.43	6.22±0.46	5.37±0.43
Utah	5.75±0.29	41.08±1.98	6.72±0.27	12.70±1.22	5.47±0.49	5.20±0.32
South America	7.25±0.29	40.53±1.69	8.06±0.92	14.39±1.91	7.03±0.83	6.45±0.14
Saltgrass (mean)	**6.96±0.32**	**43.09±0.84**	**6.83±0.26**	**11.81±0.54**	**6.30±0.16**	**5.63±0.18**
Alfalfa (as a reference)	-	-	16.48	17.15	7.20	6.91

In Table 2, we show a partial picture of the potential forage quality of our saltgrass accessions, segregated according to their geographical origins. In comparison to the genotype DE3, which had been used in previous examinations (Pasternak et al., 1993), there is a large potential in saltgrass for considerably high leafiness. It should be noted here, that leafiness was ranked among saltgrass accessions only, not against other grass species. It appeared that accessions brought from the southern regions of the US displayed significantly superior values of that parameter, although plant material with considerable leafiness can be found in other regions as well. Ash content at about 6.8% of DM was quite acceptable for forage, yet it might change much with the level of soil/water salinity (to be examined in future stages of our project). The content of crude protein (as calculated from N content) was remarkably lower than that of alfalfa. It was particularly low among accessions brought from southern US. Nevertheless, crude protein content did not correlate well

with the ME values that were quite close to that of the reference forage species, alfalfa (Table 2). ME values were consistently higher for sheep than for goat rumen.

Growth Response to Salinity

Although *D. spicata* is, in general, resistant to high levels of salinity, significant differences between ecotypes are likely to occur. Since high productivity, not only survival under salt stress is in the scope of this project, it is necessary to select for those ecotypes that are adequately productive at the level of soil salinity present at the target regions. Interesting ecotypes were grown in water culture in a greenhouse. The salinity level of the water was increased gradually during five weeks, to form six treatments (six replications in each) that differed in the levels of water salinity: 1.5, 10, 20, 30, 40, and 50 $dS \cdot m^{-1}$. Plants were weighed weekly during eight successive weeks and a curve of relative growth rate against salinity level was produced.

Those experiments provided some indications for the potential of selected ecotypes to produce biomass over a range of salinity levels, before expensive field evaluation is carried out.

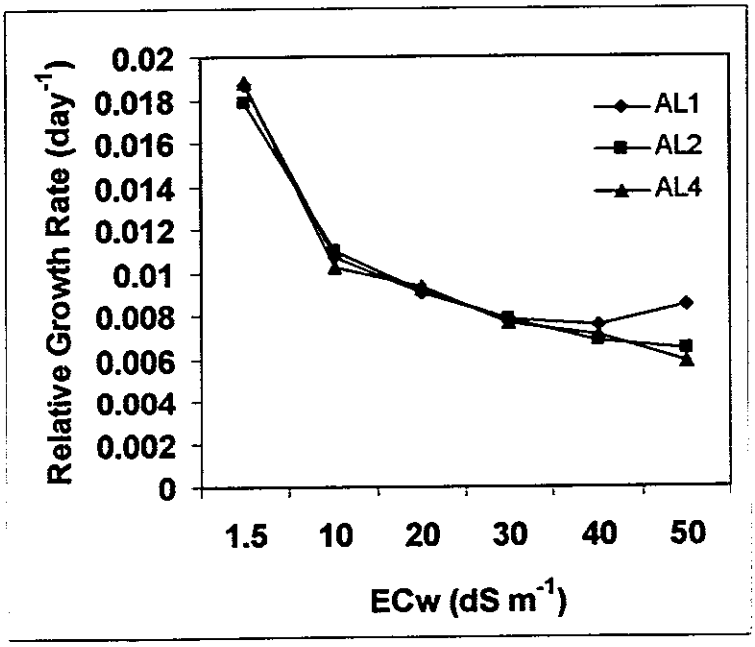


Figure 5a. Relative growth rates of three accessions from Alabama, US, grown in water culture at different levels of water salinity.

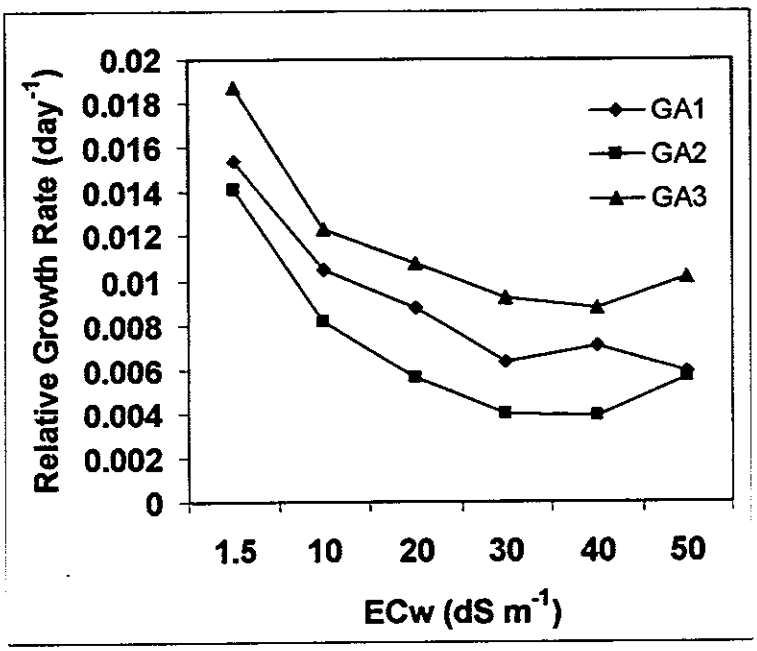


Figure 5b. Relative growth rates of three accessions from Georgia, US, grown in water culture at different levels of water salinity.

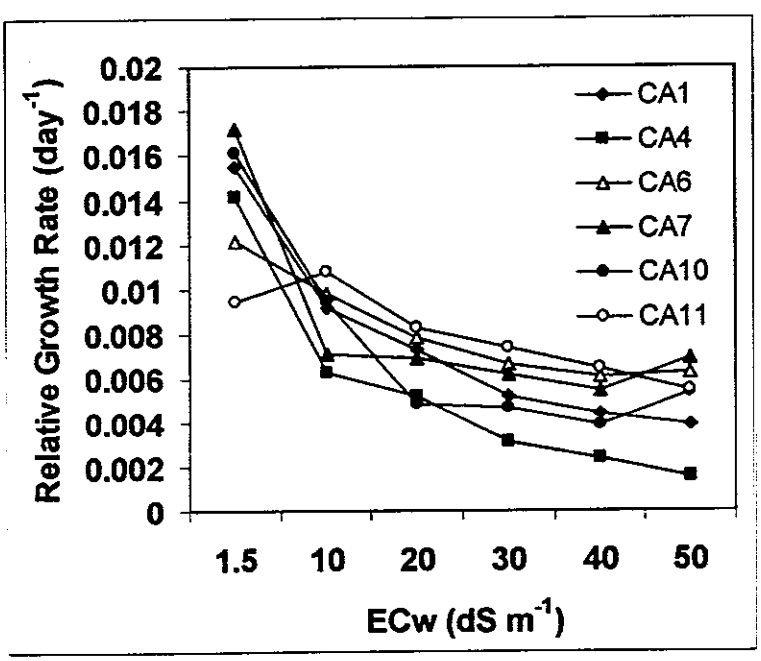


Figure 5c. Relative growth rates of six accessions from California, US, grown in water culture at different levels of water salinity. Closed and open symbols indicate for inland and coastal accessions, respectively.

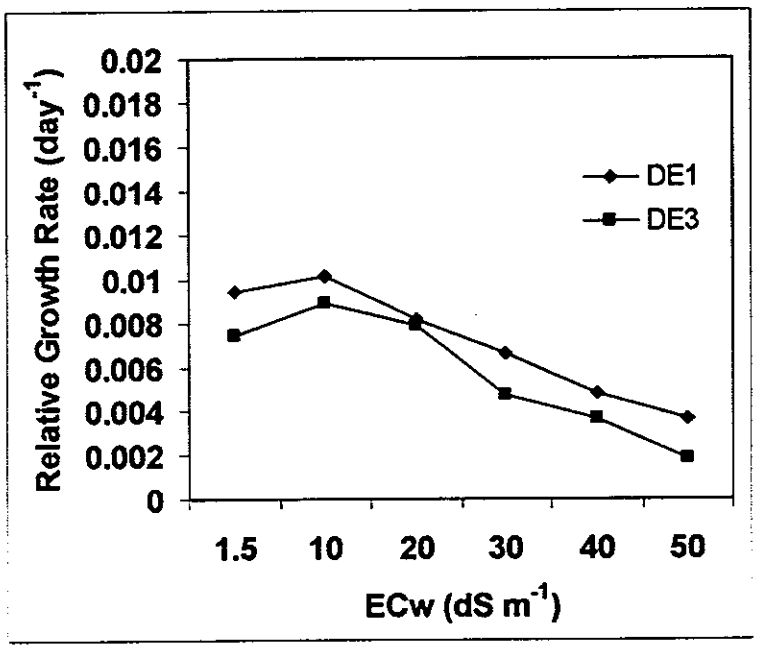


Figure 5d. Relative growth rates of two accessions from Delaware, US, grown in water culture at different levels of water salinity.

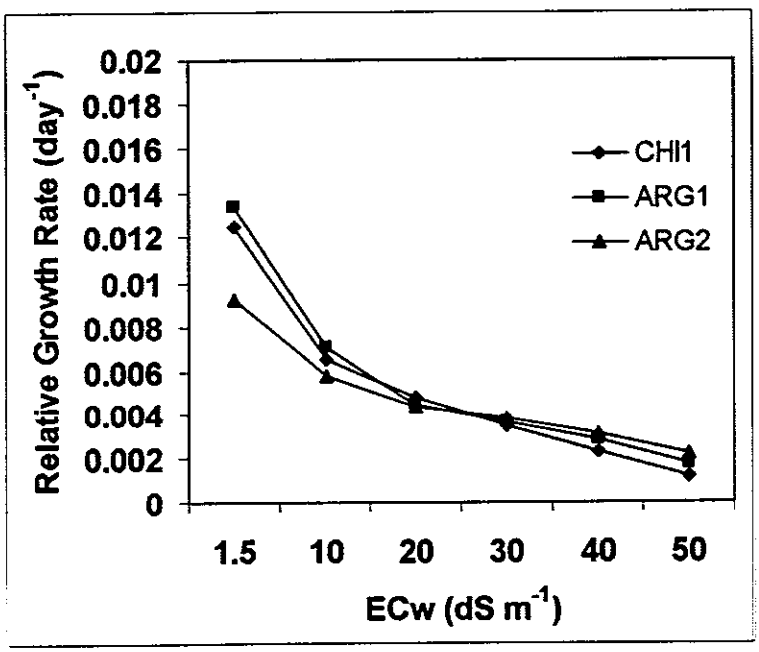


Figure 5e. Relative growth rates of three South American accessions grown in water culture at different levels of water salinity.

In most cases, RGR values declined with the increased salinity level of the water (Fig. 5), indicating that generally salt stress reduces growth and production of

saltgrass. Large differences occurred in the ability of the accessions to maintain considerable RGR at high salinity levels. All accessions from Alabama and two from the Georgia displayed significantly good performance (RGR>0.006) at high salinity levels (Fig. 5a,b). Variability among accessions from California was very large, with some advantage to coastal accessions at high salinity levels (Fig. 5c). DE3, which had been the first saltgrass genotype examined by Pasternak et al. (1993), displayed some tendency to grow better at moderate salinity levels, but RGR declined significantly at the higher salinity levels. The other Delaware accession showed a parallel behavior but lower RGR values (Fig. 5d). South American genotypes had the least RGR values in comparison to most other accessions (Fig. 5e).

Establishment of field experiments

Two small scale experiments were established this year in Israel. The first one is aimed to examine saltgrass response to NPK fertilization levels. Two accessions, GA3 and ARG1, were planted in large containers on a Perlite-2 bed. Four levels of NPK fertilizer will be tested on the background of fresh and saline water applications (1.5 and 10 $dS \cdot m^{-1}$, respectively). The amounts and mineral composition of the drainage water will be monitored. This year plants were allowed to grow and fill the volume of the containers under even conditions of fertigation (Fig. 6). The various fertigation treatments will be applied during the summer of 2003. We shall examine production performance (yield of fresh and dry matter) and effects on forage quality parameters.

Figure 6. The NPK experiment.

The second experiment is aimed at examining the response of the same two accessions to a gradient of salinity in the field (Fig. 7). Four irrigation lines (sprinklers) were put across the field, of which each supplies water at different salinity level ascending from fresh (1.5 $dS \cdot m^{-1}$) through 4, 8, and 12 $dS \cdot m^{-1}$ at the highest. The overlapping watering areas of the lines create the required salinity gradient in the soil. Fertilizer will be applied evenly through irrigation. Plants were planted in August 2002 and will achieve full coverage toward the summer of 2003, when salinity treatments are about to start. Plants will be harvested periodically when plants of the moderate salinity levels reach a certain height. Fresh and dry matter yield will be determined and plant material will be examined for forage quality parameters and accumulated for animal feed trials. At the end of the experiment, plant material from this field will be transferred to Turkmenistan to establish a similar field experiment using a similar irrigation device.

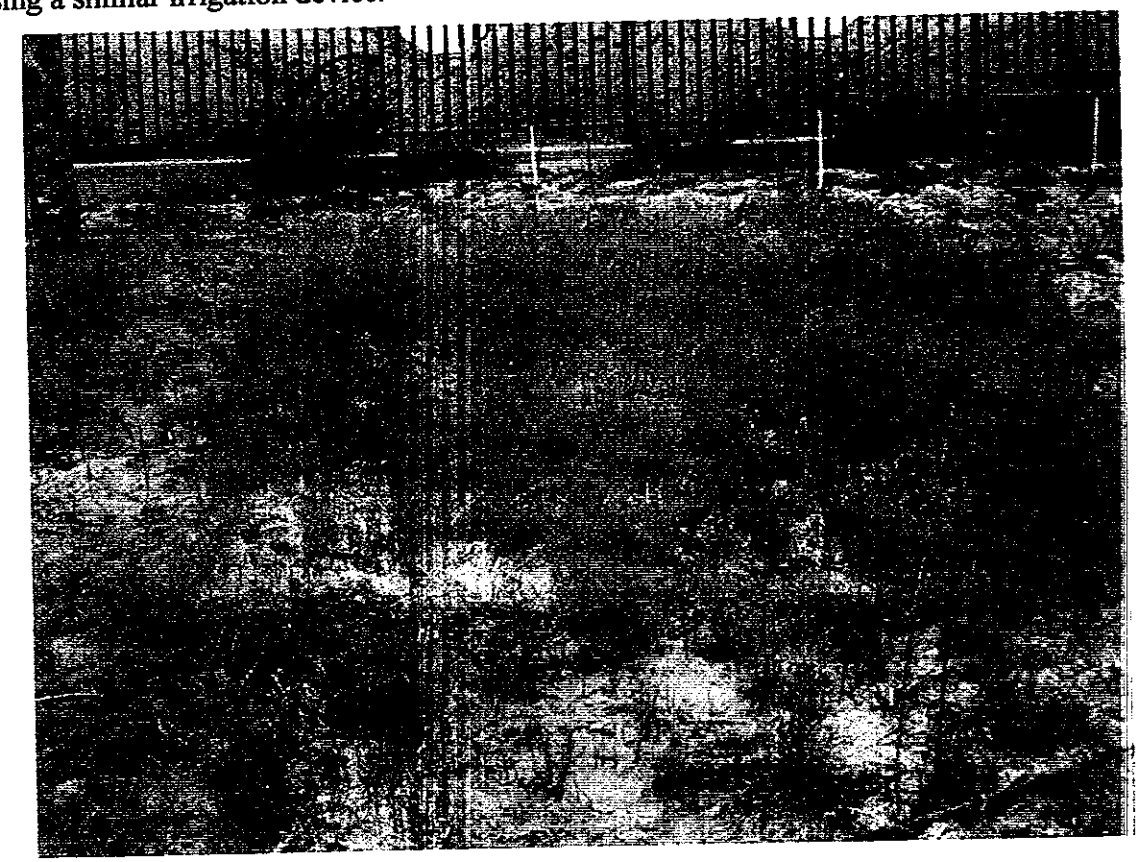


Figure 7. GA3 saltgrass accession about two months after planting in the salinity gradient experiment. Note the sprinkler lines (3 out of 4 are visible in the picture) across the field.

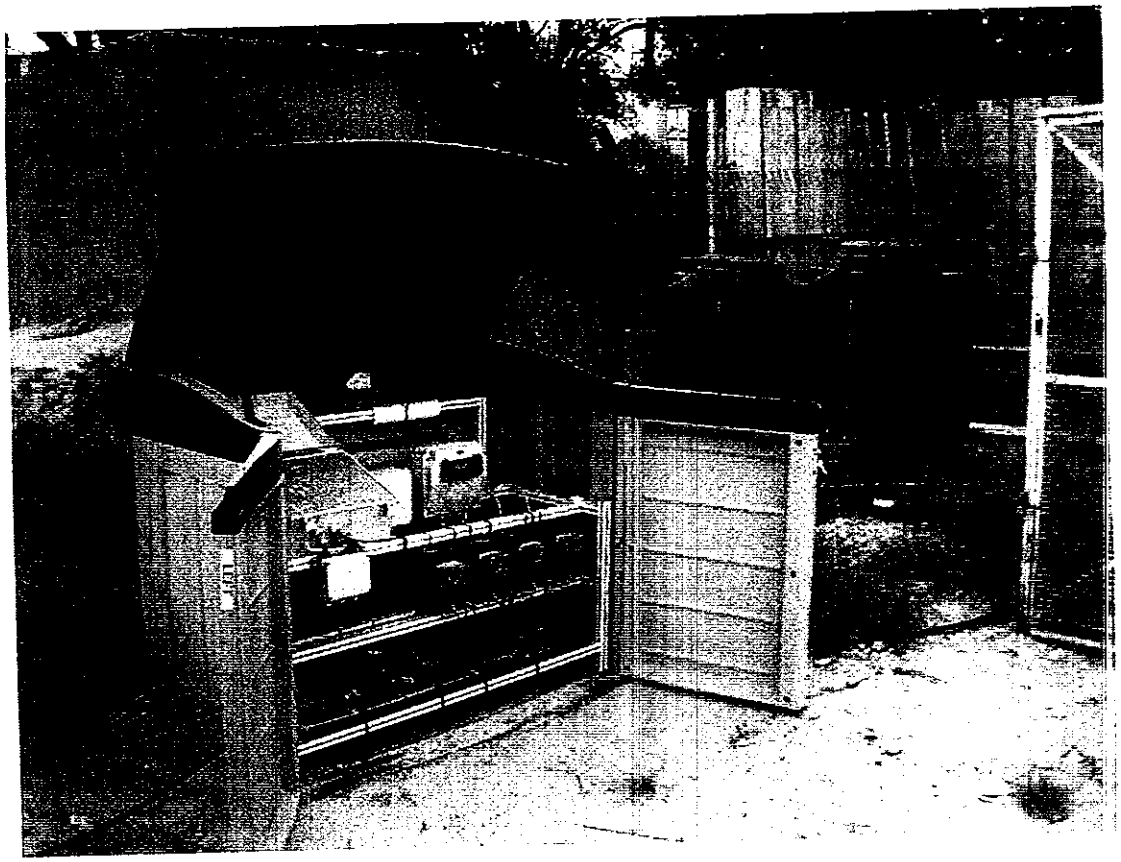


Figure 8. The irrigation system designed for the salinity gradient experiment. At the back there are two containers, one for fertilizer and the other for concentrated brine. A computer controls and monitors the operation of 4 electrical pumps, one of which injects the fertilizer and the others inject brine at the requested concentration. A similar approach will be used to design and modify a system suitable for the conditions in Turkmenistan (where water must be pumped for irrigation and electrical energy must be provided through a solar or alternative device).

Conclusive Remarks

At the end of this stage of the research, it can be stated that a considerable level of variation exists among our saltgrass accessions. Yet, the performance of the majority at increasing levels of salinity is far beyond other known forage grasses. At this stage we can say, that accessions brought from the southern coast of the US are the most promising. However, the potential of other origins, such as California, should not be neglected. Also South America should not be discarded as a potential origin of good plant material, since the number of accessions brought from there was extremely small. Therefore, we intend to widen our collection in the future by importing new

accessions from the Mexican Golf, the south Atlantic coast of the US, the Californian Pacific coast, and from South America.

The quality of the saltgrass accessions as forage is apparently promising. It may increase following appropriate selection procedures and fertigation regime. Nevertheless, forage value parameters must be further examined at saline conditions. Also, palatability and digestibility should be estimated before any conclusions are made.

Six accessions from various origins, which appeared acceptable at very early stages of research have been already brought to Turkmenistan (June 2001), planted there and their performance in two different locations is evaluated. However, the bottleneck of the project seems to lie at the propagation stage. We do not have saltgrass seeds, which are not produced in Israel (and are hardly produced at the origin countries). Therefore, we must rely on vegetative propagation, which sometimes fail to obtain the required amount of plant material. This problem slows our efforts to establish large field experiments at this stage.